Extended summary SCI Pesticides Group Meeting: Systemic Acquired Resistance

The following extended summary is based on a presentation at the meeting 'Systemic Acquired Resistance', organised by M Smith and AH Cobb on behalf of the SCI Pesticides Group and held at 14–15 Belgrave Square, London on 10 March 1998. It is entirely the responsibility of the authors and does not necessarily reflect the view of the Editorial Board of Pesticide Science.

Effects of CGA245704 on wheat physiology

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Crop growers have several options with which to prevent disease. Avoidance strategies involving cultural methods such as cultivar selection and reduced fertilizer use may be combined with direct chemical disease control through the use of fungicides. Although there is a great deal of information still to be learned about the mechanisms involved, it is recognised that Systemic Acquired Resistance (SAR) is a potentially important new technology for crop protection, not least by theoretically reducing the selection pressures that favour fungicide resistance.[1] In many plants, SAR is a long-lasting, broad-spectrum defence response induced by pathogenic infection, although the mechanisms involved remain complex and uncertain. A natural SAR reaction begins with an initial recognition of an avirulent pathogen leading to an indeterminate period of non-specific elevated resistance at multiple steps in the infection process, the duration of which varies greatly between and within plant species.[2] Initiation of SAR with an exogenous activator may give a more reliable and consistent defence suitable for commercial use.

It has been demonstrated that the Novartis compound CGA245704 (*S*-methyl benzo [1,2,3]thiadiazole-7-carbothioate) has plant activator activity in a wide range of crop plants against a number of pathogens and at 30 g AI ha^{-1} has provided good protection in winter wheat (*Triticum aestivum* L) from powdery mildew (*Blumeria graminis* Speer (formerly *Erysiphe graminis* DC)).[3] In addition, three further effects have been regularly reported with treated plants in the UK and elsewhere. When CGA245704 was applied to winter wheat between December and February, a foliar greening phase was observed. This was a transient effect and lasted for up to three months until stem extension at GSZ 32.[4] A second effect was a more erect growth habit in treated plants, particularly obvious after emergence of the flag leaf at GSZ 39. However, a later application of CGA245704, at or beyond GSZ 33, often led to chlorosis in older treated leaves. Persisting for up to one month, this symptom was unacceptable when present, but was not reported with the same frequency as the two previous symptoms. None of these symptoms has led to yield losses at harvest.

The importance of environmental factors in the generation of these symptoms was implied by a number of field reports. For example, field trials in Germany in May reported the appearance of chlorosis on treated plants when cold nights were followed by bright, hot days. Conversely, chlorosis was very seldom observed in field trials in Scotland, which may be linked to the lower photon flux densities of the Scottish winter and spring. Whilst these symptoms were routinely observed in the field, they could not be repeated in the glasshouse. An investigation was therefore initiated in 1996 which aimed to reproduce symptoms observed in the field under controlled conditions, identify the main physiological components of symptom development and establish the physiological consequences of CGA245704 treatment in winter wheat.

To establish these effects under controlled conditions, a number of variables, including cultivar choice, growth medium, plant density, nitrogen availability, temperature and photon flux density were examined. Brigadier was found to be the most sensitive of the three cultivars studied, followed by Haven and Slejpner. In an initial experiment using cv Brigadier sown at high density (*c* 4000 seeds m^{-2}), chlorosis was observed three days after treatment with field rate CGA245704 (30 g AI ha^{-1}). Whilst the other cultivars displayed the same symptoms, they did so more slowly and to a lesser extent. Chlorotic lesions were restricted to older leaves, spreading basally, and tissues eventually became necrotic. An interesting consequence of high-density sowing was that older leaves in the centre of the tray were shaded and developed fewer and smaller lesions than exposed foliage.

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Several growth media and incubation systems were examined for consistency of response in further studies. An in-vitro assay using leaf segments floating on a range of CGA245704 solutions (0–1.2 g litre^{-1}) in Petri dishes was unsuccessful, simply demonstrating that CGA245704 is a very effective carbon and nitrogen source for micro-organisms. The most successful growth medium to date to reproduce symptom development has been Silvaperl graded horticultural Perlite (expanded volcanic rock; William Sinclair Horticulture Limited, Lincoln, UK) in 12.7 cm diameter pots. These were placed in shallow troughs and watered from below, allowing control of nutrient status. Plants were grown at 20 °C under 16 h light/8 h dark regime provided by Poot Lichtenergie 400-W sodium growth lights (Schipluiden, Holland) and sprayed at GSZ 12–13, typically 17 days after sowing.

To determine the role of plant stress in symptom development, plants were exposed to a range of photon flux densities at three levels of nitrogen. Chlorosis was more apparent at 15 mg litre^{-1} nitrogen (supplied as ammonium nitrate in the solution) than at 90 or 150 mg litre^{-1}. Whilst this symptom was clearly demonstrated at 200–500 μmole photons $m^{-2}s^{-1}$ photosynthetic photon flux density (PPFD), a repeat of the experiment in a growth cabinet at 30 μmole photons $m^{-2}s^{-1}$ PPFD produced no chlorosis in either treatment. Treated tissue was found to be greener under these conditions, a typical adaptation to a low flux density. Further experiments will examine this response in more detail.

The greening response was found to be dependent on nitrogen availability. At 90 and 150 mg litre^{-1} the leaves of treated plants which emerged post-chemical application were greener and more erect than the equivalent control leaves. However, at 15 mg litre^{-1} nitrogen the leaves of both control and treated plants were similar in appearance. Chlorophyll analysis has suggested that this effect was transient.

Future studies will investigate antioxidant status and free-radical-scavenging enzyme activity to determine whether these have a role in greening and chlorosis. It is known that the activities of various enzymes in tobacco, including glutathione reductase, glutathione *S*-transferase and superoxide dismutase increased after SAR induction[5] and ascorbate peroxidase was inhibited by the plant activators salicylic acid and 2,6-dichloroisonicotinic acid. Intriguingly, this inhibition was correlated to the ability of the molecule to induce defence-related genes.[6]

Assimilate partitioning will also be examined in treated plants and the role of flux density, which seems to be important in the generation of chlorosis, will be studied in detail. Field-grown plants exhibiting the erect growth habit will be studied for enhanced lignification, possibly corresponding to SAR-related wall-bound peroxidase activity.[7] In addition, these plants will be studied for evidence of net carbon gain.

In conclusion, preliminary observations have shown that effects of CGA245704 on wheat in the field can be reliably reproduced in controlled environments using cv Brigadier as a sensitive indicator. Further work is in progress to elucidate the underlying mechanisms involved in the generation of these symptoms.

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